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RNA Viruses that Cause Hemorrhagic, Encephalitic, and Febrile Disease

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A group of small RNA viruses belonging to the families *Togaviridae*, *Bunyaviridae*, *Arenaviridae*, and *Filoviridae* cause hemorrhagic, encephalitic, or febrile disease throughout large areas of the world. All are associated with insect or rodent vectors whose interaction with humans defines the mode of disease transmission. Because these viruses occur primarily in Asia, Africa, and South America they are typically considered in the context of "tropical medicine" and are considered as "exotic viruses," both because of their unusual biology of transmission and the increased level of biohazard protection, biosafety level BL2 to BL4, required for their safe handling in the laboratory. Because they tend to occur either in underdeveloped countries, or with such low frequencies, the development of antiviral therapies has not been a commercial priority. Further, funding for studying many of these viruses is

difficult to obtain from traditional sources. Research on them is focused in a few research institutes specializing in tropical medicine. Traditionally, the United States Army has been a major contributor to such research efforts, with laboratories both in the United States and overseas in several endemic areas, and through an extramural contract/grant system to support such work. The Centers for Disease Control (CDC) also conducts significant work in this field. Summarizing the field of clinically important viral diseases in tropical medicine is beyond the scope of this chapter, and the reader is referred to major reviews in the references for further information (1-3). Where several of these diseases represent either significant health

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threats to large numbers of individuals or are useful prototype illnesses, significant work on antiviral chemotherapy has been done. The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) has, for several years, had the only major drug discovery program directed specifically at many of these viruses, with a screening capacity of 1,500 compounds per year against 11 viruses, as well as animal models for studying many of the diseases.

The task of drug development is hampered by our limited understanding of many of the diseases and the viruses that cause them, due often to the remote location of outbreaks and the inherent problems in conducting sophisticated studies in such locations. Drug discovery is constrained by the requirement of utilizing biocontainment facilities, to include BL3 and BL4 or "spacesuit" laboratories, with their associated support facilities. This has significantly limited the number of laboratories able or willing to participate in development. In spite of these difficulties, significant progress has been made in developing therapy for the viral hemorrhagic fevers and several lead compounds are now being studied for febrile and encephalitic illnesses.

VIRAL HEMORRHAGIC FEVERS

Viral hemorrhagic fevers cause severe clinical illness and represent a significant health threat in several areas of the world, as seen in overview in Table 1. They are caused by small enveloped RNA viruses transmitted by specific vectors or contact with rodent host. Their clinical presentation is similar although they are caused by members of the *Togaviridae*, *Bunyaviridae*, *Arenaviridae*, and *Filoviridae* families. Successful clinical trials of ribavirin have been completed against two of these diseases.

Bunyaviruses

The *Bunyaviridae* family comprises more than 200 named viruses divided into five genera. Many of the *Bunyaviridae* members are firmly established as human pathogens. Three of these diverse array of viruses stand out as significant global problems: Rift Valley Fever, Crimean Congo hemorrhagic fever, and hemorrhagic fever with renal syndrome (4).

Hemorrhagic Fever with Renal Syndrome

Etiologic Agent

Following the propagation of hantaan virus in *Apodemus* (5) and its adaptation to cell culture (6), the agent was identified as a bunyavirus by immunoelectron microscopy (IEM) (7-9) and described as spherical, enveloped virions, with an average diameter of 95 nm. Biochemical characterization revealed that hantaan, like other members of the *Hantavirus* genus of the family *bunyaviridae*, possesses a single-stranded RNA genome of tripartite antisense message enclosed in a ribonuclease-sensitive nucleocapsid surrounded by a lipid envelope containing two virus-specified glycoproteins (10-13). No serological relationship can be demonstrated between hantaan virus and any other member of the *Bunyaviridae* (14).

Epidemiology

Hemorrhagic fever with renal syndrome (HFRS) is a group of closely related diseases known by several synonyms (epidemic hemorrhagic fever, Churilov disease, epidemic nephritis, epidemic nephrosonephritis, hemorrhagic nephrosonephritis, Songo fever, Korean hemorrhagic fever, Far Eastern hemorrhagic fever, nephropathia epidemica, endemic benign nephropathy, virus hemorrhagic fever, muroid virus

nephropathy) (15). HFRS is caused by three of the four distinct viruses in the genus *Hantavirus* of the family *Bunyaviridae* (16,17) and is acquired by contact with chronically infected rodent hosts. Hantaan viruses, the cause of Korean hemorrhagic fever in Korea (18,19) and epidemic hemorrhagic fever (Songo fever) in China (20,21) and Japan (22), and hemorrhagic nephroso-nephritis in the Soviet Union (23-27) are associated with the rodent host *Aodemus agrarius* (striped field mouse) (5,28). Puumala virus, the cause of nephropathia epidemica in Scandinavia, is transmitted by *Clethrionomys glareolus* (bank vole) (29), and Seoul virus, the cause of a less severe form of HFRS in China and Korea, is associated with *Rattus rattus* and *Rattus norvegicus* (urban rats) (30). A severe form of HFRS is recognized across Asia including Korea (5), People's Republic of China (31,32), Japan (33), Hong Kong, Malaysia, Singapore (34), and the Eastern USSR (24-28). A milder form, nephropathia epidemica (35), occurs west of the Ural mountains in Scandinavian countries of Sweden (36), Finland (29), Norway (37), and Denmark (38), associated with puumula virus. Isolated cases of a mild form of HFRS have been reported in France (39), West Germany, Belgium (40), Scotland (41), and Italy, whereas a severe form occurs seasonally in Hungary (42), Czechoslovakia (43), Rumania (44), Bulgaria (45), Albania, Yugoslavia (46), and Greece (47,48).

Unlike other vectorborne *Bunyaviridae*, infection with hantaviruses is apparently associated with contacting chronic, asymptotically infected rodent hosts. Transmission is believed to occur via aerosolized urine or feces of rodents (49,50); infection from contact with bodily fluids of infected individuals has not been documented. It is apparent that the epidemiology of hantaviruses is intimately associated with the complex ecology of their principal vertebrate hosts. Disease in China is variously estimated from 100,000 to more than 500,000

cases per year and occurs predominantly in rural areas among farmers, foresters, and soldiers stationed in the field. It is most prominent in males (60-80%). In Korea, 1 000 cases are seen each year. The disease has two seasonal peaks: one in late fall (October through January) and a smaller peak in early summer. In addition to infection from natural sources, more than 200 laboratory-acquired infections have been noted, both in laboratories working on HFRS and among animal handlers in laboratories (due to persistently infected rats). Several infections have been traced to infected animal colonies, although the scientific community has now taken steps to control infection through testing of breeding stocks.

Clinical Features of Severe Form

The characteristic features of HFRS are a triad of fever, hemorrhagic phenomena, and renal insufficiency. Disease severity ranges from mild to grave based upon discriminators of severity, but no simple prognostic indicators of clinical severity are available on admission to predict outcome. In most patients, the clinical course can be divided into five often overlapping stages, but individual patients can be quite variable, skipping some stages entirely (16,44, 51-56). The specific diagnosis of classical HFRS depends on recognition of its characteristic multiphasic features together with an appropriate exposure history. Serologic confirmation of specific immunoglobulin M (IgM) can be made (within 8 hr) in most patients on admission [$>95\%$ in People's Republic of China (PRC) study (57)] either by indirect immunofluorescence (IF) on hantaan-infected Vero E6 cells or by an IgM enzyme immunosorbent assay (ELISA) (57,58).

Febrile Phase. A prodrome is rare. The incubation period of HFRS ranges from 1 to 4 weeks, with an average of 14 days, and extremes of 4 and 60 days. The febrile stage

TABLE 1. *Viral hemorrhagic fevers: etiologic and epidemiologic considerations*

			Epidemiologic features of involvement of humans			
	Causative agent	Vector(s)	Vertebrate host(s)	Geographical distribution	Control	Remarks
YF (urban)	YF virus—a flavivirus	<i>Aedes aegypti</i> in cities	Humans	Human populations (usually urban) in tropics of South and Central America and Africa	<i>Aedes aegypti</i> control; vaccination	Sylvan YF can spread to cities
YF (sylvan)	YF virus—a flavivirus	<i>Haemagogus</i> mosquitoes in new world; <i>Aedes</i> species in Africa	Monkeys of several genera and species	Forests and jungles of South and Central America and West, Central, and East Africa	Vaccination	Human cases sporadic and unpredictable; disease often a "silent" epizootic in forests
Dengue hemorrhagic fever	Dengue viruses of four types; flaviviruses	<i>Aedes aegypti</i>	Humans (involvement of other primates has been postulated)	Tropical and subtropical cities of Southeast Asia and Philippines	<i>Aedes aegypti</i> mosquito repellent, screens, etc.	Disease may represent an immunologic overresponse to a sequential infection with a different dengue strain

OHF	OHF virus—a flavivirus	Ticks of genus Dermacentor	Small rodents and muskrats	Omsk region of USSR; northern Rumania	People exposed in fields and wooded lands	Tick repellents and protective clothing
KFD	KFD virus—a flavivirus	Ticks of several species in genus Haemaphysalis	Monkeys (rhesus and langur) and small rodents and birds	Mysore State, India	People exposed in fields and wooded lands	Tick control; tick repellents and protective clothing
AHF	Junin virus—an arenavirus	None recognized	Small rodents; Akodon; Calomys laucha, musculus	Argentina: Northwest of Buenos Aires extending west to Province of Cordoba	Field workers at harvest time are particularly at risk	None
Bolivian hemorrhagic fever	Machupo virus—an arenavirus	None recognized	Small rodent, Calomys callosus	Beni Province of Bolivia	Residents of small, rodent-infested villages and homes, 1971 nosocomial outbreak in Cochabamba, Bolivia	Rodent control in villages
Lassa fever	Lassa virus—an arenavirus, LCM-related	None required	Small rodent, Mastomys natalensis	West Africa; Nigeria, Liberia, Sierra Leone	Residents of small rodent-infested villages; dramatic nosocomial outbreaks	None known; possibly rodent control
Crimean hemorrhagic fever	CCHF virus—a nairovirus	Ticks of several genera	Larger domestic animals implicated; also African hedgehog	Southern USSR, Bulgaria, East and West Africa	Cowhands and field workers in USSR; nosocomial outbreaks reported	Tick control relating to livestock; full isolation in patient care

continued

TABLE 1. Continued

	Causative agent	Vector(s)	Vertebrate host(s)	Geographical distribution	Epidemiologic features of involvement of humans	Control	Remarks
HFRS [Korean hemorrhagic fever hemorrhage nephrosonephritis]	Hantaan virus—a hantavirus	Not known	Small rodents: <i>Apodemus</i> , <i>Clethrionomys</i>	China, Korea; northern Eurasia to and including Scandinavia	Rural or sylvan exposure (military, forest occupations, farmers)	Rodent control in towns	Urban rats may be reservoirs
Ebola/Marburg	Ebola Marburg	Unknown	Unknown	Africa	Handling of infected primates; person to person during outbreak	Unknown	High mortality in humans

OHF, Omsk hemorrhagic fever; KFD, Kyasamur Forest disease.
From ref. 130, with permission.

begins with abrupt onset of high fever (39°C to >40°C), chills, malaise, and myalgia, followed by headache, eye pain, dizziness, and anorexia. Within 1–3 days, large extravasation of plasma into the peritoneum and retroperitoneal space results in severe back and abdominal pain. Vascular dysregulation leads to characteristic flushing of the face, neck, and chest together with conjunctival hemorrhage. High white blood cell (WBC) counts are prognostic of severe disease. During the early febrile stage, the urine may contain small amounts of albumin, which increases abruptly in the late febrile phase. This stage lasts 3–7 days. Most patients seek medical care toward the end of this phase.

Hypotensive Phase. The hypotensive stage occurs coincident with defervescence; hypotension develops abruptly, lasting from several hours to 3 days. The classical picture of shock may occur, including tachycardia, narrowed pulse pressure, hypotension, cold and clammy skin, and dulled sensorium. Purpura and mucosal bleeding from respiratory, genitourinary, and gastrointestinal tracts may occur. Massive proteinuria is accompanied by a progressive fall in urinary specific gravity.

Oliguric Phase. The oliguric stage lasts 3–7 days. Blood pressure begins to normalize, but many patients become hypertensive due to relative hypervolemia. Prolonged periods of hypertension are predictive of poor prognosis. Bleeding tendencies increase in severity as manifested by extensive purpura, mucosal hemorrhage, and cerebral hemorrhage. Urine output, already compromised to a variable degree because of renal hypoperfusion, falls to oliguric or even anuric levels associated with increasing uremia. Serum creatinine levels may increase dramatically to greater than 10 mg/dl, with blood urea nitrogen (BUN) increasing to over 200 mg/dl. Central nervous system (CNS) symptoms and pulmonary edema may occur in severe cases. Nearly 50% of deaths occur in this stage, generally associated with renal failure, pul-

monary edema, and electrolyte abnormalities.

Diuretic Phase. Clinical recovery begins with the onset of the diuretic stage, which may last for days to weeks. Diuresis may be delayed due to dehydration, electrolyte imbalance, and infection in some patients. Diuresis of 3–6 liters daily is the rule and can give rise to marked, life-threatening shifts in fluid and electrolyte balance. The convalescent stage lasts for several months and is characterized by progressive recovery of glomerular filtration rate, renal blood flow, and urine concentrating ability. In China, most patients return to light duty during this period, but anemia and hypotaenuria may persist for months to years.

Clinical Features of Mild Form (Nephropathica Epidemica)

The characteristic features of nephropathica epidemica (NE) are biphasic, consisting of fever and renal insufficiency (29). Mortality in NE is low (<1%). The incubation period of NE averages 1 month (range 3 days to 6 weeks), and a prodrome is rare. The febrile stage begins with abrupt onset of high fever (39–40°C), chills, malaise, and headache, which last 2–9 days. Between the third and fourth days, somnolence, nausea, vomiting, back pain, and occasionally joint pain appear, heralding the onset of the renal phase. Restlessness and blurred vision are seen in one-fourth of patients, whereas characteristic severe abdominal pain, sometimes diffusely localized to the right lower quadrant, occurs in some patients. Renal involvement generally appears with lysis of fever. Clinical signs are accompanied by proteinuria (100%, peaking at 1 week), oliguria (>50%), and azotemia (>85%). Elevations of serum creatinine of 2–10 mg/dl and BUN of 50–200 mg/ml may be accompanied by mild electrolyte derangements. Mild hypotension is seen in 40% of patients during the first week, but hypertension is not seen. Oliguria is short

lived and is followed by polyuria of 3–4 liters per day for 7–10 days. Hypothenuria is universal. Cylindruria, pyuria, and microscopic hematuria are seen in most patients, but gross hematuria is rare. Mild leukocytosis and thrombocytopenia occur during the renal phase of illness. With the onset of polyuria, clinical recovery begins. Patients are subjectively well within 14–17 days following onset of fever.

Prevention and Treatment of Severe Form

No prevention is currently available, although efforts to develop both conventional and recombinant vaccines are underway. Treatment consists of supportive care, with careful attention to fluid balance. Mortality of untreated disease ranges from 15% to greater than 50%, and with best supportive care without renal dialysis varies from 5% to more than 30%. In facilities with intensive care, including aggressive use of renal dialysis, a mortality rate of 2–3% is associated primarily with hemorrhage.

Experimental Therapeutics

Preclinical Studies. *In vitro* studies have shown that hantaan virus is among the most sensitive of RNA viruses to ribavirin. By way of comparison, plaque reduction 50% effective dose (ED_{50}) values for Rift Valley Fever (RVF) virus ($ED_{50} = 80 \mu\text{g/ml}$) and sandfly fever [SF (Sicilian)] virus ($ED_{50} = 77 \mu\text{g/ml}$) are substantially higher than for hantaan virus ($ED_{50} = 25 \mu\text{g/ml}$).

Early studies showed that ribavirin could prevent the appearance of hantaan antigen in the lungs of experimentally infected rodents belonging to the species *Apodemus agrarius*, the natural viral host (59). Subsequently, ribavirin has been evaluated in suckling mice infected with hantaan virus at 24 hr of age (60). These mice develop viremia, detectable viral antigen in tissues, and a clinical syndrome marked by weight loss,

depressed activity, hind limb paralysis, and ultimately death (61).

Ribavirin treatment of infected suckling mice was initiated at different stages postinoculation, with doses ranging from 0 to 100 mg/kg continued over 14 days. Treatment started at the onset of viremia on day 6 or at the appearance of viral antigen in tissues on day 10, resulted in a decrease in signs of illness, an increase in survival, and an increase in mean time to death (MTD) in mice that died. The effects of ribavirin were dose-dependent. To characterize the mechanism of protection produced by ribavirin in the suckling mouse model, a serial sacrifice study was performed employing the most promising treatment regimens: 25 mg/kg begun on day 6, and 50 mg/kg begun on day 10, with corresponding controls of placebo treatment and no treatment (60). This study established that protection was associated with decreased viremia and decreased tissue antigen in multiple organs, including liver, spleen, lung, kidney, and brain. The fluorescent antibody response, although delayed by 2 days in ribavirin-treated animals, followed a course similar to control animals. Neutralizing antibody appeared at the same time in both treated and control groups. The favorable effects of ribavirin on hantaan viral infection in suckling mice are especially impressive because drug toxicity peculiar to suckling mice limits drug doses to levels that are suboptimal for cures in other bunyavirus mouse models. Attempts to develop an adequate primate model for HFRS have not been successful.

Clinical Trials. The success of these preclinical studies prompted a clinical trial in the PRC from 1985 to 1987 (57,58,60). A prospective, randomized, double-blind, placebo-controlled clinical trial of intravenous ribavirin therapy of HFRS (33 mg/kg loading dose; 16 mg/kg every 6 hr for 4 days; 8 mg/kg every 8 hr for 3 days) was conducted in a nine-site study in Hubei Province, PRC (57). During two epidemic seasons, 244 patients met the study criteria

for analysis (enrollment within 4 days of fever onset, extended to 6 days the second season, with clinical diagnosis serologically confirmed by IgM ELISA). Statistical analysis demonstrated random assignment of patients between treatment groups. Reduction in mortality was the most important (primary) determinant of the drug efficacy. Treatment significantly reduced mortality from 10 of 118 in the placebo group to 3 of 126 in the ribavirin-treated group ($p = 0.041$ by a stratified Fisher's exact test). A stratified analysis of all valid patients entered in both years of the study showed mortality was significantly reduced among ribavirin-treated, compared to placebo-treated patients when comparisons were adjusted for baseline risk estimators of mortality [total serum protein and AST (SGOT) identified in the placebo group by logistic regression], utilizing a stepwise logistic procedure [$p = 0.02$ (two-tailed)]. This improvement in survival may be partially explained by the reduction in kidney damage seen in the drug-treated group. Ribavirin treatment decreased: maximum serum creatinine ($p = 0.05$), duration and magnitude of hypertension, fraction of patient entering oliguria ($p = 0.02$). Ribavirin therapy decreased the fraction of patients experiencing hemorrhage ($p = 0.03$). Ribavirin shortened the duration of each post-febrile clinical phase. The only significant side effect was a reversible anemia.

Ribavirin is currently an investigational new drug for HFRS and has not been licensed. Intravenous ribavirin therapy at appropriate doses has provided the first effective therapy for early treatment of HFRS in this study. Treatment reduced mortality and improved several important aspects of the clinical course. An ongoing clinical trial for the treatment of all patients is continuing among United States troops who contract the disease in Korea and Okinawa.

A similar study was also conducted in Wuhan, PRC (62) to evaluate recombinant interferon- α therapy of HFRS. Although hantaan virus is sensitive to interferon- α *in*

vitro (63), mortality was not reduced and no significant treatment effect was found at doses of 1×10^7 U per day for 5 days, although indications of reduction in bleeding tendencies were noted. Higher doses were not tested due to dose-limiting toxicity.

Prevention and Treatment of NE

No prevention is currently available, and treatment consists of supportive care with renal dialysis when required. Ribavirin has not been evaluated in this form of the disease.

Rift Valley Fever

Etiologic Agent

RVF, an old-world phlebovirus, shares with other members of the *Bunyaviridae* family a lipid-enveloped spherical structure with a diameter of 90–120 nm, with 5–10 nm surface projections. They mature by budding into the cisternae of the Golgi region. The virion contains two surface glycoproteins, G1 and G2, which are the hemagglutinin (HA) and neutralization targets. It has a negative-stranded segmented tripartite genome composed of three RNA species designated large (L), medium (M), which codes for G1 and G2, and small (S), which codes for the nucleocapsid protein and may be of "ambisense" polarity.

Epidemiology

RVF, distributed throughout sub-Saharan Africa, causes serious and occasionally fatal infections in humans (64–68). Epizootics have been documented in Kenya, South Africa, Namibia, Mozambique, Tanzania, Uganda, Zimbabwe, Sudan, Central African Republic, Rhodesia, and Egypt as widely spaced outbreaks during the rainy season that infrequently extend to the next, then disappear for several years. RVF first

appeared in Egypt in 1977 when an estimated 200,000 cases occurred with 598 reported deaths. Most cases had a typical febrile illness, but predominant complications included hemorrhagic fever, encephalitis, and exudative retinitis. RVF is also a significant pathogen of sheep and cattle. The virus has been isolated from several genera of mosquitoes. *Culex* and *Aedes* have been implicated during epizootics. Recent findings from Kenya that *Aedes* mosquitoes emerging from flooded depressions called "damboes" are already infected with virus provide strong evidence for transovarial transmission (3,69). RVF can also be transmitted by aerosol.

Clinical Features

Human infection is usually (95%) a severe but self-limiting disease. In less than 5% of cases, disabling or life-threatening complications can occur. Following an incubation period of 2–6 days, back and muscle pain, anorexia, and incapacitating prostration occur. Physical findings are limited to conjunctival and pharyngeal injection. Epistaxis may occur, and a "saddle-back" fever is not uncommon. Initial leukocytosis is followed by leukopenia, composed mainly of lymphocytes. The illness usually lasts 2–5 days, and recovery is without complications (2,65,69,70).

A hemorrhagic form may develop in 1% of patients by the second to fourth day but cannot be predicted on admission. These patients develop petechiae, ecchymosis, hematemesis, melena, and bleeding of the gums. Liver function tests, including prothrombin, bilirubin, transaminase, and alkaline phosphatase, are elevated. Patients become jaundiced and die in shock. The prognosis is poor for patients with hemorrhagic disease and approaches 50% mortality. Recovery is slow but without sequelae (65,67).

Encephalitis can occur 5–10 days after the acute febrile episode, with a presenta-

tion of headache, meningismus, vertigo, confusion, hallucinations, and recrudescence of fever. The case fatality rate is unknown but has been estimated at 10% (3). Recovery is slow but without sequelae.

Ocular complications occur late in otherwise asymptomatic patients 1–3 weeks after acute illness, with brisk onset of impairment of visual acuity resulting from retinal hemorrhages, exudates, macular and paramacular edema, vasculitis, retinitis, and vascular occlusion. Lesions were bilateral in 50% of patients, and 40–50% suffer some permanent loss of visual acuity (69).

Prevention and Treatment

No specific treatment exists, and uncomplicated disease is best managed by symptomatic treatment and observation. Hemorrhagic complications should be managed by standard techniques, but anticoagulation therapy of disseminated intravascular coagulation may be ill-advised due to virus-induced liver damage (69).

Experimental Therapeutics: Preclinical Studies

Experimental infections of RVF in mice or hamsters result in death due to hepatitis on days 4–6 in virtually all animals (71,72). Ribavirin is very effective prophylactically and shows a bell-shaped dose response curve with 100% survival produced by 100 mg/kg/day (71). Punta Toro serves as a lower biohazard model for RVF in routine antiviral screening in the USAMRIID program. Ribavirin treatment of Punta-Toro-virus infected hamsters with 100 mg/kg/day on days 0–4 increased survival from 10% to 90% and MTD from 5 to over 45 days (72). Similar results are obtained in a murine model routinely used for testing antiviral drugs *in vivo* (73). Rhesus monkeys challenged with RVF virus intravenously (IV) and treated with ribavirin initially 2 hr after virus inoculation by the intramuscular (IM)

route (50 mg/kg loading dose followed by 10 mg/kg tid) had significantly lower viremia ($p < 0.001$) compared to those of sham-treated control monkeys. All infected monkeys had serum neutralizing antibody titers of 1:80 or greater by day 7, even though two of four ribavirin-treated monkeys were not detectably viremic (74). Interferon in this same model is also protective (75). Poly(ICLC) is effective in the murine model, as are combinations of ribavirin and poly(ICLC).

Crimean-Congo Hemorrhagic Fever

Etiologic Agent

Caused by a nairovirus genus of the family *Bunyaviridae*, the properties of Crimean-Congo hemorrhagic fever (CCHF) are similar to those of Rift Valley Fever.

Epidemiology

CCHF, transmitted by ticks, occurs over a wide area of the world, mainly in steppe, savannah, semi-desert, and foothill biotopes where the tick parasites are present on both domestic and wild animals in a large area of East and West Africa. Human disease has been documented as sporadic focal infection in the Union of Soviet Socialist Republics, Bulgaria, Pakistan, Dubai, Iraq, the Emirate of Sharjah, Zaire, Uganda, Mauritania, Burkina Faso, Upper Volta, Union of South Africa, Ethiopia, Nigeria, Senegal, Greece, Tanzania, Namibia, and the People's Republic of China (3). The distribution of virus is the second widest of all arboviruses and its distribution, based on virus isolation or serological studies, is from southern Europe to China. CCHF virus has been isolated from many species of ticks, and members of three genera have been shown to be capable of transmitting infection, but ticks of the genus *Hyalomma* have always been regarded as the main vec-

tors (76). Disease is related to the interaction of ticks and their vertebrate hosts, which is complex. Secondary infections are common in a hospital setting. A nosocomial outbreak in Tygerberg Hospital illustrates the potential where an index case resulted in eight infections and two deaths. Typically, a severely ill patient presents to the hospital with severe hemorrhagic disease, but the diagnosis is not made on admission so proper isolation procedures are not initiated until after infection of medical personnel has occurred through contact with blood. The diagnosis is first suggested when 3-7 days later medical personnel involved in direct care, laboratory workers, patients in nearby beds, and/or close family members present with hemorrhagic disease and often a high mortality rate.

Clinical Features

The disease in the Soviet Union has been described in detail (24,77-79). The incubation period is estimated to be 3-12 days based on recall of tick exposure, and 3-6 days in nosocomially acquired cases. Onset is abrupt in virtually all cases, with severe headache, fever over 39°C, myalgia, weakness, anorexia, back and abdominal pain, and nausea, often accompanied by vomiting. There is hyperemia, most notable on the face, mucous membranes, and upper part of the body. The illness generally follows a biphasic course, early nonspecific symptoms being followed after the sixth day of illness by hemorrhage from the nose, mouth, and gastrointestinal tract. The appearance of large ecchymotic areas on the limbs is a particularly noticeable feature. Large purpuric areas sometimes occur. Most cases are apathetic or obtunded with halting speech; dizziness and mild meningeal signs are common. Severe cases will be delirious or comatose. Shock and death from circulatory collapse often occur. Laboratory findings include leukopenia and thrombocytopenia to 30,000/mm³. Hemato-

crit is normal or elevated on presentation but falls with hemorrhage to less than half of normal values in severe cases. The case fatality rate has reached as high as 30–50% in nosocomial outbreaks (69,80–82) but typically is 9–40%. Differences in clinical course are described, with the Middle Eastern form yielding extensive nosocomial secondary and even tertiary spread, and there is a high prevalence of severe liver involvement with clotting abnormalities.

Prevention and Treatment

There is no proven specific therapy. Convalescent immune plasma may be useful, but controlled studies have not been reported. Hospitalization, including careful attention to proper isolation procedures to avoid potentially devastating nosocomial spread, can be effectively implemented without the requirement for specialized treatment facilities. Laboratory and nursing staff must exercise care to avoid generation of aerosols. Recommendations for handling of cases have been published (83).

Experimental Therapeutics

Preclinical Studies. Development of animal models has proven difficult. Available models utilize suckling mice infected on days 1–5, with the notable exception of one Russian report of a lethal adult mouse model, in which successful ribavirin therapy is reported. Ribavirin appears to be uniformly effective *in vitro* (84) and increases both the number of survivors and MTD in suckling mouse models (G. Tignor and B. Shope, unpublished observations).

Clinical Studies. During an outbreak of CCHF at the Tygerberg hospital (80,81), a large 2,000-bed teaching hospital near Cape Town South Africa, six of nine inoculation contacts were given ribavirin prophylactically (83). One patient had a mild clinical course, whereas the other five developed neither clinical CCHF nor antibodies to the

virus. Although two of three needle contacts not treated with ribavirin developed a severe clinical course, one needle contact and 42 proven blood contacts who did not receive ribavirin also did not show any signs of clinical disease. Thus, no firm conclusions could be drawn about the prophylactic use of the drug. No obvious treatment failures were observed, but an opportunity to evaluate efficacy of the drug does not appear to have been present, because of the low attack rate among patients with blood contact. Due to the toxicity of interferon (1.7×10^7 U), which was also tested, ribavirin remains the best available prospect for therapy.

Arenaviruses

Arenaviruses include four human pathogens of which three produce hemorrhagic disease (4). The virulent new-world arenaviruses predominantly cause disease in men and are acquired in rural areas in the fall, when agricultural products are harvested. Transmission is thought to occur by contact with chronically infected rodent hosts (84). In West Africa, the arenavirus Lassa fever has also been found to be sensitive to ribavirin (85,86). Over the last several years, primate models have been developed to study the pathogenesis and treatment of each of these viruses. Studies in experimental animal models of guinea pigs and monkeys infected with either Machupo, Junin, or Lassa viruses have shown both prophylactic and therapeutic efficacy of ribavirin.

Lassa Fever

Etiologic Agent

Lassa virus is an enveloped, single-stranded, bisegmented RNA virus classified in the family *Arenaviridae*. Negative-staining electron microscopy (EM) shows the presence of pleomorphic particles rang-

ing from 80 to 150 nm. The envelope is formed by budding from the plasma membrane of infected cells. The virus contains three major structural proteins: two glycoproteins and a nucleocapsid.

Epidemiology

Lassa fever is a severe disease of West Africa, caused by Lassa virus, one of two pathogenic old-world arenaviruses. The natural host is the multimammate rat *Mastomys natalensis*, which is ubiquitous across sub-Saharan Africa and occupies ecological niches in both forest and savannah regions. Evidence suggests only one species is involved in infections (84). They are infected and shed high levels of virus throughout their life. The epidemiology of all arenaviruses is defined by the factors that determine maintenance and spread of the agents among rodents, which in turn spread virus into the environment. This African rodent, which lives in close association with humans, is found in and around most dwellings, the most important locations for transmission of the virus to humans. Under natural conditions, infection occurs via contact with *M. natalensis* or its excreta within the household (83). Naturally occurring infections, often associated with subsequent nosocomial outbreaks, have been recognized in Nigeria, Sierra Leone, and Liberia (1) and less frequently in Guinea, Senegal, Mali, and the Central African Republic (1,88). Several cases have been imported into the United States and Europe (89,90), but secondary transmissions have not been documented. Person-to-person spread required close personal contact or contact with blood or excreta. Proper barrier infection control procedures appear adequate to control nosocomial spread. Of infections, 70–90% result in mild or asymptomatic infections. Overall mortality of recognized infections is 1–2%, a major revision of initial impressions based on nosocomial outbreaks (91). The mortal-

ity of hospitalized patients is 15–20%. In West Africa, 50,000–150,000 infections occur each year and account for 5–15% of febrile illness.

Clinical Features

The clinical spectrum of Lassa fever is quite variable, and the ratio of infection to illness is 9–26%. After an incubation period of 1–3 weeks, Lassa fever presents as an insidious onset of progressive fever, malaise, and myalgia, and a sore throat. At time of hospitalization, patients are toxic and mildly hypotensive. Pain is seen in the joints and lower back, along with headache, and a nonproductive cough. Retrosternal or epigastric pain, vomiting, diarrhea, and abdominal discomfort are also common. Frank bleeding tendencies may develop during the course of the illness, particularly in severely ill patients. Illness may last 3–4 weeks. Poor prognostic signs include sustained fever, bleeding diathesis, severe hypotension or shock, coma, and convulsions (89,92–100). Various degrees of permanent sensorineural deafness result in 25% of patients. Adverse prognostic factors are AST elevations above 150 IU/liter and high levels of viremia during hospitalization. The latter are not measurable in time to be clinically useful (83).

Prevention and Treatment

Treatment is supportive and may require intensive care facilities. Attention must be paid to fluid and electrolyte balance, maintenance of blood pressure and circulatory volume, and control of seizures (83).

Experimental Therapeutics

Preclinical Studies. Infection of rhesus monkeys with 10^6 PFU of Lassa virus resulted in six of 10 deaths between 10 and 14 days after inoculation. The six lethally in-

fectured animals had viremia titers that significantly exceeded 10^4 PFU/ml, whereas none of the surviving monkeys developed viremia in excess of this apparently critical titer. These results are quite similar to the human disease in which admission viremia is a predictor of survival (101). In four monkeys treated with ribavirin (50 mg/kg loading dose, followed by 10 mg/kg three times daily), from day 0 through day 18, the onset of detectable viremia was delayed until day 7, and peak viremia titers were significantly lower (10^2 PFU/ml) than those of surviving control monkeys. Clinical illness was mild and brief in monkeys that received ribavirin beginning on day 0. Some monkeys exhibited no clinical signs at all, whereas others became only slightly depressed and developed a minimal facial rash during the second week. All treated monkeys survived, even when ribavirin therapy was delayed until day 5. Four monkeys receiving ribavirin first on day 5 experienced a moderately severe disease course. However, all monkeys treated therapeutically with ribavirin by day 5 eventually recovered with no evident sequelae, and viremia titers never reached the critical level. Thus, therapeutic administration of ribavirin can reverse the hemorrhagic component of the disease (85). In cynomolgus monkeys, 13/14 untreated monkeys died, whereas ribavirin treatment begun at 0, 4, and 7 days resulted in 0/4, 0/4, and 4/8 deaths (102). Combination therapy with immune plasma possessing a log neutralizing index of greater than 2 was also evaluated with very promising results (102). These studies led to a clinical trial of ribavirin treatment of Lassa fever in Africa.

Clinical Trials. Ribavirin chemotherapeutic trials in Lassa fever patients were initiated in 1979 in Sierra Leone. An open, non-placebo controlled trial of oral ribavirin was begun first, followed by a trial of higher dose intravenous ribavirin (2 g loading dose then 1 g every 6 hr for 4 days, then 0.5 g every 8 hr for 6 days) in an attempt to improve survival over that observed with oral ribavirin. The mortality of patients

hospitalized with acute Lassa fever in Sierra Leone is 16%, based on data from over 400 Lassa fever cases seen in two hospitals during the 3-year period preceding the drug study. Patients with Lassa fever may be subdivided by two indicators of mortality risk: admission levels of AST (SGOT) and viremia. An admission serum AST over 150 carries a risk of mortality of 50%, whereas an admission viremia over $10^{3.6}$ TCID₅₀/ml carries a mortality rate of 76%. This high-risk group allowed for demonstration of drug efficacy, based solely on survival. Patients treated with IV ribavirin who had an admission viremia over $10^{3.6}$ TCID₅₀/ml had a mortality of 32% compared with 76% in patients who were untreated. The time of initiation of treatment, in relationship to the clinical course of the disease, was also determined retrospectively. Patients who began treatment early, before day 7 of clinical signs, had a significantly improved chance of survival, compared to late initiation of treatment. This observation of the need for early initiation of treatment is shared with all antiviral drugs currently in use (101).

Argentine Hemorrhagic Fever and Bolivian Hemorrhagic Fever

Etiologic Agent

Bolivian hemorrhagic fever is caused by Machupo virus. Argentine hemorrhagic fever (AHF) is caused by Junin virus. Both are members of *Arenaviridae*, with properties similar to Lassa virus.

Epidemiology

Bolivian hemorrhagic fever is localized to the sparsely populated tropical savannah in the northeast of Bolivia. As with all arenaviruses, the epidemiology is influenced by the rodent host *Calomys callousus*, which causes focal house-related out-

breaks. Rodent control programs have resulted in no outbreaks since 1974 although isolated cases are still seen (J. Miaztegui, unpublished observations). AHF occurs in the farmland of the humid pampa, predominantly in Buenos Aires and Cordoba Provinces. AHF is an occupational disease predominantly affecting men harvesting grain crops, principally maize. The primary rodent vector is *Calomys muscullinus*. Its density fluctuates widely over a 3–5-year period and correlates with human disease rates.

Clinical Features

Insidious onset of fever, headache, asthenia, and myalgia after an incubation period averaging 7–14 days characterizes this syndrome. Mild or subclinical infection is unusual in contrast to Lassa and lymphocytic choriomeningitis (LCM) virus infection. Back pain, epigastric discomfort, retroorbital pain, photophobia, constipation, and occasionally mild diarrhea appear during the following 2–5 days. During this interval, patients are progressively toxic and lethargic with flushed face and trunk, conjunctival inflammation, and by the fourth to the sixth day of illness, fine petechiae in the axillary region. There may be lymphadenopathy and a palatine or faucial exanthem comprised of petechiae or fine vesicles. Pharyngitis is uncommon. Dysesthesia of the skin may be severe. Panleukopenia (to 1,000/mm³) and thrombocytopenia (to 25,000/mm³) are always present. Protein may appear in the urine along with cylindrical casts and erythrocytes.

At the end of approximately 1 week of fever and just prior to its resolution by lysis, patients begin to improve clinically (approximately 60%) or enter into a clinical crisis consisting of either a bleeding-shock phase lasting no more than 3 days or a neurological syndrome that may last up to 1 week. Mixed forms are commonly noted in AHF. The bleeding disease is heralded by

petechiae, a rising hematocrit, and increasing proteinuria. If not treated promptly and, in some instances, irrespective of intervention, hypotension and disturbances in consciousness ensue. When clinical shock appears, the prognosis is grave. Pulmonary edema is a common complication, and bleeding from the gastrointestinal tract and mucous membranes is always evident.

In both Argentina and Bolivia, case fatality rates are approximately 15%, with neurologic disease more common in the former country and hemorrhagic disease predominant in the latter. Patients surviving this disease require several weeks for convalescence, and paroxysmal and orthostatic hypotension, asthenia, and mild anorexia are common (84,103).

Prevention and Treatment

Administration of specific antibody (2 U of immune plasma) has been proven effective (mortality reduced from 16% to 1%) if given within 8 days in a double-blind trial of normal and convalescent plasma (104).

Experimental Therapeutics

Preclinical Studies with Machupo. Junin, the causative agent of Argentine hemorrhagic fever, is sensitive to ribavirin *in vitro* (71,105), as is closely related to Machupo, which causes Bolivian hemorrhagic fever (72,86). The therapeutic potential of ribavirin was first evaluated in the treatment of Machupo-infected guinea pigs. Only 1/15 untreated animals survived, whereas 12/15 ribavirin-treated animals survived. Treatment was significant at the $p < 0.001$ level (72). Ribavirin was next evaluated using a rhesus monkey model (106–108). Sham-treated virus control monkeys reached peak viremia by days 12–14 and began to die. Treatment of individual monkeys was initiated at the time of onset of fever with 25 mg/kg and continued every 8 hr for 10 days. Quite remarkably, viremia

responses of treated monkeys were lower by day 7 compared to sham-treated control monkeys and virtually undetectable by day 10. Regardless of the time of initial treatment or regimen of therapy, ribavirin prevented death during the acute phase of illness. The late neurological syndrome, seen in 20% of infected, untreated control monkeys, however, was not prevented in 4/5 treated animals. It is presumed that the late neurological phase of the disease results from the inability of ribavirin to reach the CNS in sufficient concentration (72).

Preclinical Studies with Junin. Prophylactic as well as therapeutic studies of ribavirin in Junin virus infection of rhesus macaques also have been performed (109). Sham-treated control animals infected with $10^{4.8}$ plaque-forming units (PFU) of the highly lethal Espindola strain of Junin virus showed 100% mortality with a classical hemorrhagic diathesis during the third and fourth weeks. Monkeys administered ribavirin on a prophylactic schedule (60 mg/kg/day for 4 days, 30 mg/kg/day for 3.5 days, then 15 mg/kg/day for 11 days) seroconverted, but failed to develop viremia or clinical signs of illness. In animals receiving ribavirin therapeutically beginning on day 6 postinfection (60 mg/kg/day for 1.5 days, then 15 mg/kg/day for 14 days), viremia was detected until the time of drug administration, then disappeared for the duration of observation. These animals had early signs of bleeding abnormalities (redness around eyes, circular ocular redness, dried blood in nose) and decreased platelet counts prior to onset of therapy. Treatment resulted in reversal of clinical disease including resolution of hemorrhage, petechiae, and restoration of platelet counts. As occurred in Machupo-treated animals, however, a late-onset neurological syndrome appeared in all animals, and was fatal in two of three. Similar studies in *Callithrix jacchus* (cotton-eared marmoset) revealed similar results (110).

Clinical Trials. A clinical trial of ribavirin

is currently underway at the Instituto Nacional de Estudios sobre Virosis Hemorragicas, Pergamino, Argentina. A specific treatment is available for AHF, which consists of the administration of immune plasma with defined neutralizing antibody titers. However, this treatment is only effective when given within the first 8 days after onset of symptoms. Immune plasma therapy is not effective after 8 days of illness (104,111,112). An open study was therefore undertaken in this group of late patients to determine tolerance and antiviral activity. Six patients were treated during the epidemic season. Ribavirin treatment resulted in significant reduction in viremia and a drop in endogenous interferon (high interferon levels are indicators of poor prognosis) and an increase in average time to death (113). This prompted a placebo controlled study that has enrolled 18 patients to date.

Filoviridae

The *Filoviridae* family of viruses was first identified in 1967 when an outbreak associated with laboratory workers harvesting tissues from wild caught African green monkeys developed Marburg disease. The two members of this family, Ebola and Marburg, cause the most severe of the viral hemorrhagic fevers (VHF), but outbreaks have been rare and research must be conducted in BL 4 containment laboratories.

Ebola/Marburg

Etiologic Agent

The morphology of Ebola and Marburg is unique. Particles range from 130 to 14,000 nm in length but are more uniform in diameter (80 nm). The nucleocapsid is surrounded with a lipid envelope and contains a single-stranded RNA. Five polypeptides are associated with the virion (54).

Epidemiology

Marburg and Ebola represent two recently recognized viral hemorrhagic fevers of Africa. Marburg was first recognized in 1967 when seven deaths, which occurred among 31 cases in Germany and Yugoslavia, were traced to direct contact with blood, organs, or tissue culture cells from a group of African green monkeys caught in Uganda. In 1976, outbreaks of Ebola occurred in Sudan and Zaire with over 500 cases and 400 deaths, respectively. The disease occurs in all age groups, but with a predominance in adults. Transmission from person to person requires close contact, particularly with blood or body fluids. The method of maintenance in nature is not known.

Clinical Features

The clinical picture of Marburg and Ebola is indistinguishable. Following an incubation period of 4–16 days (average of 7 days), clinical illness begins suddenly with fever, malaise, headache, and myalgia followed by nausea, vomiting, and watery diarrhea. A maculopapular rash appears between days 5 and 7 and is most marked on the buttocks, trunk, and outer aspects of the upper arms, and conjunctivitis is common. Liver function is impaired by the second week of illness, but jaundice is not observed. Renal damage has been observed. Disseminated intravascular coagulopathy is a major feature of the disease, and bleeding occurs in the majority of cases, mainly from the gastrointestinal tract. Case fatality rates from 29% to 89% in outbreaks have been reported (114–116).

Experimental Therapeutics: Preclinical Studies

Ebola is not significantly inhibited *in vitro* by ribavirin in Vero, MRC-5, or FRhL

cells. Ribavirin prophylaxis (20 mg/kg/day) of Ebola utilizing the guinea pig model with either the Zaire or Sudan strain resulted in no change in survivors but a significant prolongation of MTD with the Sudan strain. A study utilizing 100 PFU of Zaire strain of Ebola in cynomolgus monkeys was conducted. Animals were treated with ribavirin at 50 mg/kg loading dose, 4 hr prior to infection, followed by 20 mg/kg tid until time of death. No significant effect of the drug was seen (H. Lupton and J. Moe, unpublished observations).

Flaviridae

Two flaviviruses cause viral hemorrhagic fevers (VHFs) and are widely distributed in nature. Yellow fever (YF) often presents as a VHF, whereas Dengue fever is primarily a febrile illness with outbreaks or isolated cases of VHF.

Yellow Fever

Etiologic Agent

As all flaviviruses, YF virus is a positive, single-stranded RNA virus with a spherical, host-cell-derived lipid envelope. The structural proteins consist of a major glycosylated envelope protein (E) exposed on the virion surface that contains most immunologic determinates. A small nonglycosylated envelope protein (M) is not exposed on the surface, and a nonglycosylated nucleocapsid protein (C) is found in association with the viral RNA. The morphology of all flaviviruses is quite similar, being spherical with a mean diameter of 43 nm (37–50 nm), a unit membrane envelope with surface projections, and a 30-nm diameter core. The entire genomic RNA has been sequenced for the 17D and Asibi (vaccine parent) strain.

Epidemiology

YF occurs throughout much of tropical South America and sub-Saharan Africa. The epidemiology of YF is explained by different transmission cycles of the virus among humans, mosquitoes, and monkeys. The vector mosquito, which belongs to one of several species, becomes infected by feeding on a viremic host (human or monkey) and then transmits the virus to another susceptible host. YF occurs in two major forms, urban and jungle (or sylvatic) with very different results. Sylvatic (jungle) YF circulates in a cycle involving nonhuman primates and forest or canopy mosquitoes. In Africa, the infection is not usually fatal to nonhuman primates, whereas in South America it is frequently fatal, a useful clue in identifying areas of viral transmission. In this area, YF is endemic with a year-round transmission cycle between monkeys and mosquitoes. Humans are infected when exposed to the enzootic cycle, and sporadic cases occur on a continuous basis. In the savanna surrounding the forest zone, YF is endemic. Human infections may occur at varying frequencies as vector populations expand during the rainy season. In this emergence zone, epidemics may occur, involving both monkey-to-human and inter-human transmission by sylvatic vectors. These epidemics are often characterized by focal outbreaks separated by areas without human cases. Urban YF, the oldest described viral hemorrhagic fever, is capable of explosive epidemics and still constitutes an important cause of viral hemorrhagic fevers. Urban YF is transmitted in a human-mosquito-human cycle involving domestic *Aedes aegypti*. The potential geographic distribution of urban YF is the range of *Aedes aegypti*, which includes Africa, South America, Central America, the Caribbean, and the Gulf coast of the United States. Disease incidence is modified by the presence of existing antibody, either through natural infection or by vaccination. The last major urban outbreak in a major

city in the Americas occurred in Rio de Janeiro in 1928 and 1929. The outbreak in Ethiopia in 1960–62 involved over 100,000 cases and 30,000 deaths. An outbreak in Nigeria in 1987, although not fully described, involved over 30,000 cases.

Clinical Features

YF is an acute infectious disease. The incubation period is usually 3–6 days following the bite of an infected mosquito. The clinical spectrum varies from very mild, nonspecific febrile illness to a fulminating, often fatal disease with pathognomonic features. The mild form is characterized by sudden onset of fever and headache without other symptoms, lasting 48 hr or less. The clinical diagnosis is almost impossible in most settings and must rely on serological diagnosis. In other patients, fever is higher, the headache severe, and there is myalgia, slight albuminuria, and bradycardia in relation to the height of fever (Faget's sign). The illness lasts several days with uneventful recovery (1). The so-called classical YF is characterized by a biphasic disease course. The febrile phase is characterized by abrupt onset of fever (39–40°C), chills, severe headache, lumbosacral pain, and general myalgia. The patient appears distressed and anxious, the conjunctiva is congested, and the face and neck are flushed. The tongue is reddened at the tip and edges, and the breath is foul-smelling. Anorexia, nausea, vomiting, and minor gingival hemorrhage or epistaxis may occur. Despite a persistent or rising temperature, the pulse may fall (Faget's sign). Proteinuria is minor initially but can become marked on day 3–4. This syndrome, lasting 3–4 days, corresponds to the period of viremia. This is followed by a remission with defervescence and improvement in the general condition of the patient, which lasts only a few to 24 hr. This is followed by a period of intoxication, the hepatorenal phase, characterized by rising temperature, reappearance of

symptoms with more frequent vomiting, epigastric pain, prostration, and the appearance of jaundice. Prolongation of the clotting, prothrombin, and partial prothrombin times is marked in patients with jaundice. Total and conjugated serum bilirubin levels can be very high. Serum AST and ALT levels are markedly elevated in all icteric patients, and hypoglycemia has been noted with severe liver damage. Bleeding diathesis is manifested by coffee-grounds hematemesis, melena, metroohagia, petechiae, ecchymosis, and diffuse oozing from the mucus membrane. Dehydration results from vomiting and insensible losses. Renal dysfunction is marked by a sudden increase in albuminuria and decreased urinary output. Death usually occurs on day 7-10 and is preceded by deepening jaundice, hemorrhages, rising pulse, hypotension, oliguria, and azotemia. Hypothermia, agitated delirium, intractable hiccups, stupor, and coma are terminal events. Death is largely attributable either to an early fulminating hemorrhagic fever syndrome with hepatitis and clinical jaundice or to a later renal tubular lesion with renal insufficiency reminiscent of that seen in HFRS. Convalescence is often prolonged, but complete recovery of the liver function usually occurs. An atypical, fulminant form occurs with death on the second or third day without hepatic or renal signs (reviewed in detail in refs. 1 and 117-119).

Prevention and Treatment

YF 17D is a safe and efficacious live attenuated viral vaccine prepared from infected chicken embryos under standards developed by the World Health Organization. Travel to endemic areas requires vaccination, which is valid for 10 years although immunity is probably life-long. Serious adverse reactions are extremely uncommon. Fewer than 10% of vaccinees experience headache and malaise. An immune response can be demonstrated in 95%

of recipients within 7-10 days. Vector control in urban areas directed against *Aedes aegypti* should include elimination of breeding places, which is difficult in many locations due to local practices, and the use of insecticides and larvacides. Such measures are only effective in coordination with vector surveillance programs. Despite the effectiveness of mosquito eradication programs and the availability of a safe and effective vaccine, sporadic epidemics of YF still occur in South America and Africa.

Experimental Therapeutics: Preclinical Studies

YF is sensitive to ribavirin *in vitro*, but, comparing YF sensitivity to other viruses that can be successfully treated with ribavirin in primates or humans, three strains of YF are 10- to 20-fold less sensitive in two lines of Vero cells than Lassa, HFRS, RVF, and sandfly fever (SF) (J. W. Huggins, unpublished observations). Because of differences in drug uptake and metabolism between cell lines, sensitivity comparisons must utilize the same cell for valid comparisons. This may explain why a series of primate experiments has yielded equivocal results. No acceptable rodent model is available. YF in rhesus monkeys produces a fulminate disease with a condensed disease course. This prompted a study with the well-characterized lethal model of Dakar 1279 strain of YF in rhesus monkeys (120) to evaluate ribavirin, utilizing the optimum primate dosing schedule (85). Two groups of eight rhesus monkeys were infected with 1×10^3 PFU of Dakar 1279 strain of YF and treated simultaneously with ribavirin (30 mg/kg loading 10 mg/kg t.i.d. for 7 days). No effect on either survival, MTD, or viremia titers was seen between the two groups. It is postulated that the relative insensitivity of YF to ribavirin may result from tissue levels of drug below those required to inhibit viral replication. Ribavirin hematopoietic toxicity in mon-

keys precluded testing significantly higher doses (J.W. Huggins, P.B. Jahrling, C.J. Peters, and T.M. Cosgriff, unpublished observations).

Dengue Fever

Etiologic Agent

Four serologically distinct types of Dengue fever can be recognized (DEN 1-4). See section YF for a description of physical properties. Differences exist in the molecular weight of the structural proteins. DEN 1 and 3 form a subcomplex defined by monoclonal antibodies.

Epidemiology

Dengue fever, which had been advancing for several years toward the United States from Mexico and the Caribbean, crossed the border into Texas in 1980, making dengue outbreaks possible in areas of the United States where suitable vectors exist. Since 1977, hundreds of cases have been imported into the United States by travelers from the Caribbean (117). Dengue exists as four distinct serotypes (DEN 1-4) transmitted by *Aedes* mosquitoes, principally in tropical areas of Asia, Oceania, Africa, Australia, and the Americas. Dengue is a prevalent health problem in Southeast Asia, the Caribbean, Central America, northern South America, and Africa (1). Hundreds of thousands of cases occur each year in epidemic or endemic form around the world. Outbreaks have involved over 1 million individuals, with attack rates during epidemics in focal areas of high transmission reaching 50-90% (1). During the 1978 DEN 1 epidemic in Puerto Rico, 13% of the island population was infected (121). Dengue exists in a human-mosquito-human cycle, with *Aedes aegypti* being the most important vector. All four types can coexist in the same area. Protection against homotypic reinfection is complete and probably life-

long, but cross-protection between dengue types lasts less than 12 weeks. In Southeast Asia, children are infected by all four serotypes in childhood. The background of immunity of human populations determines the incidence and age distribution of infections.

Clinical Features

Dengue virus can produce two types of disease: classical dengue and dengue hemorrhagic fever (DHF). The clinical features of classical or uncomplicated dengue fever frequently depend on the age and gender of the patient, and whether it is an initial or secondary (other serotype) dengue infection. Infants and young children may have an undifferentiated febrile disease with maculopapular rash, an acute respiratory illness, or a gastrointestinal illness. Older children and adults infected with dengue for the first time will display more classical signs. In a typical case, after an incubation of 2-7 days, the disease begins abruptly with high fever, frontal headache or retroorbital pain, retrobulbar pain, and lumbosacral aching pain. Fever may be sustained for 6-7 days or be biphasic (saddle-back). Initial symptoms are followed by generalized myalgia or bone pain that increases in severity, anorexia, nausea, vomiting, weakness, and prostration. The pulse rate may be slow in relation to the fever. A rash may appear or reappear after defervescence (day 3-5) and be maculopapular or morbilliform in nature. Generalized lymphadenopathy may occur. The peripheral WBC count is depressed, and the platelet count may fall to less than 100,000/mm³. Hemorrhagic phenomena are noted in a few cases and include petechiae, epistaxis, intestinal bleeding, menorrhagia, and a positive tourniquet test (reviewed in refs. 1 and 117). Myocarditis and various neurologic disorders have been associated with dengue fever. Central neurologic disorders appear to be more common in DHF than in clas-

sic dengue. Convalescence may be prolonged, with generalized weakness, depression, bradycardia, and ventricular extrasystoles (1).

A more severe form of dengue recognized during outbreaks is DHF. It is generally agreed that DHF is an immunologically mediated disease, as first proposed by Halstead (122). It is proposed that nonneutralizing antibodies, naturally acquired by previous dengue infection or passively acquired as maternal antibody, enhance *in vivo* replication in mononuclear phagocytes and lymphatic tissues. Increased replication of the virus in these cells may be associated with a secondary reaction in the host's attempt to eliminate dengue-infected cells, resulting in immune-mediated disease and shock. This concept is supported by the observation that DHF rarely occurs in primary dengue infection. Typical cases are characterized by four major clinical manifestations: high fever, hemorrhagic phenomena, hepatomegaly, and often circulatory failure. Moderate to marked thrombocytopenia with concurrent hemoconcentration is a finding that differentiates DHF from dengue. Two gradations are recognized, dengue hemorrhagic fever without shock (DHF) and dengue shock syndrome (DSS) that adds shock to DHF. The World Health Organization (WHO) has established guidelines to differentiate DHF/DSS from dengue. Typical cases are characterized by four major manifestations: high fever, hemorrhagic phenomena, hepatomegaly, and often circulatory failure. Moderate to marked thrombocytopenia with concurrent hemoconcentration is a distinct clinical laboratory finding that differentiates DHF from dengue, including dengue with hemorrhagic manifestations. A grading system has been established by the WHO and grading has been found clinically useful in DHF epidemics in children in Southeast Asia and the Western Pacific region, but its usefulness in adults is not fully established. The disease is initially similar to classical dengue, with a high continuous fever lasting

2-7 days. Hemorrhagic manifestations include, as a minimum, a positive tourniquet test and any of the following: petechiae, purpura, ecchymosis, epistaxis, gum bleeding, hematemesis, and/or melena. The liver is usually palpable early in the febrile phase. Patients usually recover spontaneously or after fluid and electrolyte therapy. If signs of shock are present, the disease is classified as DSS. Severe cases, after 2-5 days, rapidly progress with prostration and signs of shock (restlessness, irritability, cold clammy extremities, peripheral cyanosis, and narrowed pulse pressure). Patients in shock are in danger of dying if appropriate therapy is not given promptly. The duration of shock is short, and patients may die within 12-24 hr or recover rapidly following appropriate antishock therapy (1,117,123).

Prevention and Treatment

The major pathophysiological abnormality seen in DHF/DSS is an acute increase in vascular permeability that leads to leakage of plasma. Hypovolemic shock, as a consequence of a critical level of plasma loss, leads to tissue anoxia, metabolic acidosis, and death, if uncorrected. In most cases, early and effective replacement of lost plasma results in a favorable outcome. The consistent finding that a drop in platelet count usually signals the onset of plasma leakage is of great diagnostic and prognostic value. There is no specific antiviral therapy, but symptomatic and supportive measures are effective (see ref. 124 for complete recommendations).

Experimental Therapeutics: Preclinical Studies

DEN 1-4 are sensitive to ribavirin *in vitro*. Using a lethal murine intracranial (IC) model of DEN 2, Halstead et al. (125) were able to demonstrate activity of a lipophilic

derivative of ribavirin thought to have improved capability to cross the blood-brain barrier, both on survival and MTD. Ribavirin was not effective, but it has been established that ribavirin does not cross the blood-brain barrier in significant quantities. Useful primate models that mimic the human disease do not exist at present; however, viremia in primates can be reproducibly measured utilizing mosquito cells (peak average viremia of 10^5 PFU/ml), prompting a ribavirin trial for suppression of viremia (126). Treatment with an optimum ribavirin schedule in a blinded, placebo-controlled primate study resulted in no suppression of viremia compared to controls.

ENCEPHALITIS

Encephalitis is the serious outcome of a number of viral illnesses that usually cause inapparent or febrile illness but with a varying frequency of patients who develop encephalitis, ranging from mild, with no sequelae, to fatal. All are associated with insect vectors (Table 2). It is especially difficult to develop antiviral drugs against the encephalitic forms because of problems with drug penetration through the blood-brain barrier to the site of viral replication. Prospects for antiviral chemotherapy are uncertain, and no successful trials have been conducted.

Flaviridae

California Serogroup Viruses

Etiologic Agent

LaCross, snowshoe hare, Jamestown Canyon, and California encephalitis virus are members of the California serogroup of the genus *Bunyavirus*, family *Bunyaviridae*. Properties are similar to Rift Valley Fever.

Epidemiology

LaCross is the most frequently reported arboviral encephalitis in North America. All of the viruses are focal and are associated with culicine mosquitoes, usually *Aedes* species.

Clinical Features

LaCross infection may be inapparent or a mild febrile illness following a 1-week incubation period. LaCross encephalitis is a disease of children. Onset is sudden, with fever and headache, followed within 12–14 hr by seizures. Convulsions are present in 50% of cases. The acute illness lasts typically 7 days with gradual recovery. The case fatality rate is 0.5%. Seizure disorders are the main sequelae of LaCross.

Jamestown Canyon encephalitis, in contrast, is usually a disease of adults. Prodromal fever and respiratory illness are followed by signs of meningitis or encephalitis (2).

Prevention and Treatment

No specific antiviral therapy is available.

Japanese Encephalitis

Etiologic Agent

Japanese encephalitis (JE), a flavivirus, is a member of the West Nile antigenic group that includes Saint Louis encephalitis, Murry Valley encephalitis, West Nile, Rocio, and Ilheus. Properties of JE are similar to those of other flaviviruses.

Epidemiology

JE is a public health problem of major concern in Asia, Southeast Asia, and the Indian subcontinent. JE is endemic in Ja-

pan, the eastern Union of Soviet Socialist Republics, Korea, China, Indo-China, Indonesia, and India. In terms of morbidity and mortality, JE is by far the most important of the arboviral encephalitides. The incidence has decreased in recent years in Japan, Korea, and Taiwan through vaccination and vector control, but thousands of cases are seen annually in Thailand. The principal amplification cycle for JE involves pigs in a transmission cycle involving *Culex* and *Aedes spp.* mosquitoes and domestic animals, birds, bats, and reptiles. Two patterns of disease occurrence are observed based on the biology of the insect vector. In temperate zones, explosive outbreaks are associated with seasonal increases in vector populations. In subtropical and tropical regions, cases occur throughout the year. JE produces a high inapparent-to-apparent infection ratio of 25–500 infections to each case of encephalitis. The mortality among cases with encephalitis is 20–50%, with permanent sequelae in many survivors (2).

Clinical Features

Clinical manifestations of JE vary from asymptomatic infection to a fulminant course leading to death. Following an incubation of 5–15 days, illness is manifested by a febrile headache syndrome, aseptic meningitis, or encephalitis. Severe encephalitis begins with a 2–4-day long phase of headache, fever, chills, anorexia, nausea and vomiting, dizziness, and drowsiness of rapid onset. In children, abdominal pain and diarrhea are common. This is followed by nuchal rigidity, photophobia, altered states of consciousness, hyperexcitability, and varying objective neurological signs (1). Death occurs on the fifth to ninth day or during a more prolonged course with pulmonary complications (1). A poor prognosis is associated with respiratory dysfunction, positive Babinski signs, frequent

or prolonged seizures, prolonged fever, and albuminuria.

Prevention and Treatment

The current vaccines were produced in Japan from the Nakayama-NIH strain, and because of antigenic variation there are questions about its effectiveness against current wild strains of JE, although a recent study demonstrates efficacy in Thailand (127). A live attenuated strain is currently undergoing evaluation in the People's Republic of China (Y. Yu, unpublished observations), and the WHO has called for new efforts to select relevant strains for development of new vaccines. No specific therapy is available.

Experimental Therapeutics: Preclinical Studies

Most antiviral drugs that inhibit JE *in vitro* do not cross the blood-brain barrier. Several natural products, however, appear to be protective in a prophylactic murine model and will be evaluated further (J.W. Huggins, M. Ussery, B.J. Gabrielsen, M. Hollingshead, and B. Shannon, screening data from USAMRIID antiviral drug development program).

Saint Louis Encephalitis

Etiologic Agent

Saint Louis encephalitis (SLE) is a member of the West Nile antigenic subgroup. Properties of SLE are similar to those of other flaviviruses.

Epidemiology

SLE occurs in endemic and epidemic form through the Americas and is the most important arboviral disease in North Amer-

TABLE 2. CNS Arthropod-borne viruses that cause acute infection and encephalitis

Virus	Taxonomic group	Mode of transmission	Geographic distribution	Disease in domestic livestock
Viruses principally associated with the encephalitis syndrome; epidemic and endemic				
EEE	<i>Togaviridae, alphavirus</i>	Mosquito	Eastern North America, Caribbean, South America	Equines, penned pheasants
WEE	<i>Togaviridae, alphavirus</i>	Mosquito	Western North America, South America	Equines
VEE	<i>Togaviridae, alphavirus</i>	Mosquito, possibly other modes (see text)	Florida, Central and South America	Equines
St. Louis encephalitis	<i>Togaviridae, flavivirus</i>	Mosquito	North America, Caribbean, Central and South America	None
JE	<i>Togaviridae, flavivirus</i>	Mosquito	East, Southeast Asia; India	Equines, swine
Rocio encephalitis	<i>Togaviridae, flavivirus</i>	Mosquito	Brazil	None
Murray Valley encephalitis	<i>Togaviridae, flavivirus</i>	Mosquito	Australia	(Equines) ^a
California encephalitis	<i>Bunyaviridae, California serogroup</i>	Mosquito	North America	None
and La Crosse	<i>Togaviridae, flavivirus</i>	Tick, ingestion of milk	Europe, USSR	None
TBE: Russian spring-summer and Central European encephalitis	<i>Togaviridae, flavivirus</i>	Tick	British Isles	Sheep, equines, cows
Louping ill	<i>Togaviridae, flavivirus</i>	Tick	Eastern North America	None
Powassan				

Viruses principally associated with other syndromes, but occasionally causing encephalitis; epidemic and endemic

Sindbis (febrile illness with rash)	<i>Togaviridae, alphavirus</i>	Mosquito	Africa, Europe	None
West Nile (febrile illness with rash)	<i>Togaviridae, flavivirus</i>	Mosquito	Africa, Middle East	(Equines) ^a
YF (hemorrhagic fever)	<i>Togaviridae, flavivirus</i>	Mosquito	Africa, tropical America	None
Rift Valley fever (febrile illness, hemorrhagic fever)	<i>Bunyaviridae, phlebotomus fever group</i>	Mosquito, direct contact	Africa	Sheep, cows, goats
Colorado tick fever (febrile illness)	<i>Reoviridae, orbivirus</i>	Tick	Western North America	None
Tick-borne hemorrhagic fevers				
KFD	<i>Togaviridae, flavivirus</i>	Tick	India	None
OHF	<i>Togaviridae, flavivirus</i>	Tick	Central Asia	None
CCHF	<i>Bunyaviridae, hantavirus</i>	Tick	Eastern Europe, USSR, Africa	None

Rare and sporadic infections associated with encephalitis

Semliki Forest ^b	<i>Togaviridae, alphavirus</i>	Mosquito	Africa, Southeast Asia	(Equines) ^a
Ilheus	<i>Togaviridae, flavivirus</i>	Mosquito	South America	None
Negishi	<i>Togaviridae, flavivirus</i>	Tick	Japan	None
Langa ^b	<i>Togaviridae, flavivirus</i>	Tick	Asia	None
Thogoto	<i>Bunyaviridae</i>	Tick	Africa	None

^aDisease suspected but not well documented.

^bEncephalitis recorded in laboratory infections or experimental infections of cancer patients only; significance in naturally acquired infections unknown.

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ica. SLE is closely related antigenically to JE in Asia and Murry Valley encephalitis in Australia. Since first recognized in 1933, there have been numerous outbreaks in the western United States (Pacific coast states, primarily California), Texas, the Ohio-Mississippi Valley, Kansas, Colorado, and Florida. Two vectors have been implicated: In the western United States, *Culex tarsalis*, and in the eastern United States *Culex pipiens*. The largest outbreak occurred in 1975, with over 2,000 recognized cases. SLE occurs in epidemic form at approximately 10-year intervals, and attack rates from 1 to 800 per 100,000 population. The disease appears in July with peak incidence in August and September (1,117).

Clinical Features

The clinical picture of SLE ranges from inapparent infection to fulminant encephalitis and death. Three clinical syndromes are described: encephalitis, aseptic meningitis, and febrile headache. The severity of illness increases with increasing age, and persons over 60 are at increased risk (1). The incubation period is 4–32 days. The syndrome of febrile headache presents as an acute febrile illness with headache, frequently accompanied by nausea and vomiting. Onset is characterized by general malaise, chilliness, anorexia, nausea, myalgia, and sore throat or cough. No signs of meningeal irritation or localized neurologic abnormalities are found. The aseptic meningitis presentation is one of an acute febrile illness associated with acute or subacute meningeal signs (a stiff neck). The syndrome of encephalitis includes presentations of meningoencephalitis and encephalomyelitis. It is characterized by altered levels of consciousness, abnormal reflexes, tremor, and signs of thalamic, brain stem, and cerebellar dysfunction (1,117). A prolonged period of convalescence from a disorder called "convalescent fatigue syn-

drome" occurs in 30–50% of cases, lasting up to 3 years. In 20% of patients, symptoms of altered gait and speech disturbances, sensor motor impairment, psychoneurotic complaints, and tremors persist for extended periods.

Prevention and Treatment

Treatment is supportive, and no effective antiviral therapy exists.

Tick-Borne Encephalitis

Etiologic Agent

The tick-borne encephalitis (TBE) is a flavivirus belonging to the TBE virus complex, which consists of six members that cause human disease: TBE, Omnsk hemorrhagic fever, Kyasanur Forest disease, Negishi, Powassan, and louping-ill. There are two subtypes of TBE: Russian spring-summer encephalitis and Central European encephalitis, which differ in their tick vector and clinical expression. TBE shares the properties of other flaviviruses except for its resistance to acidic pH, a feature that makes the virus resistant to gastric acid and allows infection from ingesting contaminated milk.

Epidemiology

TBE occurs in western and eastern Europe (Austria, Denmark, Finland, East and West Germany, Hungary, Poland, Sweden, Yugoslavia, Czechoslovakia), the Soviet Union, and China in a pattern corresponding to the Ixodid tick vector. The virus is maintained in nature by a cycle involving ticks and several wild rodent vectors. *Ixodes ricinus* is responsible for transmission of the Central European encephalitis in Europe, whereas *Ixodes persulcatus* is responsible for Russian spring-summer en-

cephalitis. Other tick species are also implicated in areas that do not support *Ixodes* ticks. Infection of goats, cattle, and sheep results of shedding of virus into the milk, and inactivation by pasteurization requires the relatively high temperature of 65°C for 30 min. The epidemiology of TBE is greatly influenced by whether the disease is tick-borne or milk-borne. Cases that are tick-borne tend to be sporadic and are influenced by occupations that increase exposure to ticks. The incidence is highest spring through early fall, corresponding to periods of tick activity. Milk-borne outbreaks tend to involve whole families and are determined by milk consumption patterns (1,117).

Clinical Features

The incubation period for TBE is 7–14 days, but the clinical course differs between the two strains. The Far East form (Russian spring-summer encephalitis) is more severe, with a case fatality rate of 20%. Onset is more often gradual than acute, with a prodromal phase including fever, headache, anorexia, vomiting, and photophobia. This is followed by a stiff neck, sensorial changes, visual disturbances, and variable neurological dysfunction. In fatal cases, death occurs within 7 days. Disease is more severe in children than adults. Neurologic sequelae occur in 30–60% of survivors, especially residual flaccid paralysis of the shoulder girdle and arms. The central European form is milder, with a case fatality rate of 1–5%. The typical disease is biphasic. The first phase is nonspecific and grippé-like, lasting approximately 1 week followed by a 1–3-day remission. The second phase begins abruptly and may take the form of benign meningitis or, in more severe cases, encephalitis. Approximately 20% of survivors have sequelae, which tend to be minor (1,117).

Prevention and Treatment

A formalin-inactivated vaccine is used in the Soviet Union, and a joint Austrian-British developed vaccine is available in Europe, but is only covered by an investigational new drug (IND) in the United States. Vaccination is recommended for high-risk occupations, as is avoidance of ticks by the use of tick repellants. Unpasteurized milk should be avoided. Treatment is supportive, and no specific antiviral therapy exists.

Alphaviruses

*Eastern Equine Encephalitis/
Western Equine Encephalitis/
Venezuelan Equine Encephalitis*

Etiologic Agent

Alphaviruses have a similar uniform and spherical appearance (50–65 nm), with a somewhat fuzzy outer surface that is icosahedral, with surface spikes composed of glycoproteins. The viral particle contains a nucleocapsid that ranges from 28 to 49 nm with a single-stranded infectious RNA of 4.1×10^6 daltons (2).

Epidemiology

Eastern equine encephalitis (EEE) is maintained in a natural transmission cycle between *Culiseta melanura* mosquitoes and birds in swampy and forested areas of New Jersey and Massachusetts. For transmission to humans, it is necessary for the virus to become established in *Aedes spp.* mosquitoes before human and equine cases occur. Western equine encephalitis (WEE) is maintained in nature by *Culex tarsalis* and *Culiseta melanura* and wild passerine birds in the western United States. Venezuelan equine encephalitis (VEE) is maintained in nature by a cycle involving *Culex spp.* and

Aedes aegypti in a mosquito-rodent-mosquito cycle. Seasonal periods of peak transmission of VEE coincide with peak rainfall (2).

Clinical Features

EEE is clinically the most severe encephalitis in North America. Onset of illness is usually rapid with high fever, vomiting, stiff neck, and drowsiness. Coma can occur by the second day. Children commonly manifest edema, either generalized, facial, or periorbital. Paralysis are common during the acute phase, and EEE seems to produce a greater disturbance in autonomic functions than other togaviral encephalitides. Case fatality rates vary from 50% to 75% of symptomatic patients. Up to 30% of patients surviving the acute infection will have neurologic sequelae, often of a severe nature, requiring permanent institutional care. Inapparent infections and milder clinical forms have been described.

The incubation period for WEE is 5–10 days, and the clinical symptoms observed depend on the age of the patient. A large number of asymptomatic infections or undifferentiated febrile illnesses occur. Illness usually begins with headache, followed rapidly by fever, which can be so high as to be life-threatening. Various manifestations of altered sensorium can progress to coma. Most infants will suffer convulsions. Most adults, even with a severe clinical course, will recover completely if death does not occur. Children under 1 year of age frequently suffer permanent sequelae ranging from minimal brain dysfunction to epilepsy or severe psychomotor disorders.

Very few people infected with VEE virus will have inapparent infection, but only a small percentage develop neurologic involvement. Many patients will have an undifferentiated febrile illness but, more typically, exhibit an influenza-like illness. Symptoms are sudden onset with fevers, chills, myalgia, generalized malaise, head-

ache, nausea, and vomiting. Lumbosacral pain is a frequent complaint, and occasionally patients will also complain of sore throat and diarrhea. Some patients with mild CNS involvement will show lethargy, somnolence, or even mild confusion but do not develop seizures or other localizing signs. VEE infection rarely progresses to encephalitis in adults and is most common in children under 15 years of age. Case fatality rates are highest in children 5 years of age and under (35%) and decrease to less than 10% for older children and young adults. Patients who survive VEE may have permanent neurologic sequelae, but this outcome is less likely with VEE than encephalitis associated with other togaviruses (e.g., EEE) (1,117).

Prevention and Treatment

Treatment is supportive. No effective antiviral therapy is known. VEE is part of the USAMRIID drug discovery program and Poly(ICLC) is effective early in the infection in the murine and primate model. Ribavirin and its triacetate are not effective, however (J.W. Huggins and USAMRIID Antiviral Drug Screening Program).

PRIMARY FEBRILE ILLNESS

Undifferentiated febrile illness is characteristic of the majority of infections caused by arboviruses (Table 3). Dengue causes a significant number of febrile cases each year and is reviewed in the section of VHF because of its significant complication. Sandfly fever, although not a serious clinical disease, is included because a ribavirin prophylactic study has been conducted.

Bunyaviridae

Rift Valley Fever

Rift Valley Fever causes a febrile disease in 95% of all cases but is reviewed in the

TABLE 3. Etiologic and epidemiologic features of undifferentiated arbovirus fevers

Fever	Virus type	Principal vector(s)	Vertebrate host(s)	Geographic distribution	Human epidemiologic features
Dengue	Flavivirus (Group B)	<i>Aedes aegypti</i> , <i>Ae. albopictus</i>	Humans	Tropics and subtropics; old and new world	Varies from continuous endemic to sporadic epidemic pattern based on human-Aedes population cycles
West Nile	Flavivirus (Group B)	<i>Culex</i> mosquitoes	Birds	Africa, Mediterranean basin, central Asia	Continuously endemic (tropics) to annual epidemic in Mediterranean climates
Phlebotomus	Phlebovirus	<i>Phlebotomus</i> , <i>Lutzomyia</i> sandflies	Wild rodents?	Southern Europe, Africa, central Asia, tropical America	Annual seasonal transmission to children; epidemic whenever large numbers of susceptible adults introduced; forest-associated infections in American tropics
Rift Valley	Phlebovirus	<i>Aedes</i> , <i>Culex</i> mosquitoes	Large wild and domestic animals	Egypt, sub-Saharan Africa	Sporadic transmission to humans; many contact infections during livestock epizootics
Chikungunya	Alphavirus (Group A)	<i>Aedes aegypti</i> , <i>Ae. africanus</i> , <i>Ae. fuscifer</i>	Humans, monkeys?	Africa, southern Asia, Philippines	Basically similar to dengue
O'nyong-nyong	Alphavirus (Group A)	<i>Anopheles funestus</i> , <i>An. gambiae</i>	Humans	East Africa	Singular massive epidemic; malaria control may affect pattern
Mayaro	Alphavirus (Group A)	<i>Haemagogus</i> mosquitoes	Monkeys and/or rodents?	Tropical South America	Endemic, forest-associated infection; localized outbreaks during forest destruction
Ross River	Alphavirus (Group A)	<i>Aedes vigilax</i> , <i>Ae. polynesiensis</i> , <i>Culex annulirostris</i>	Wild mammals, humans?	Australia, New Guinea, Oceania	Endemic to epidemic, depending on mosquito abundance and immunity level in human population
Colorado tick	Orbivirus	<i>Dermacentor andersoni</i> ticks	Ground squirrels, chipmunks	Rocky Mountains of North America	Sporadic and focal summer infections during recreational and occupational activity

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section on viral hemorrhagic fevers because of its sequelae.

Naples and Sicilian Sandfly Fever

Etiologic Agent

Sandfly fever (SF) is an old-world *Phlebotomus*; its properties are similar to RVF.

Epidemiology

Naples and Sicilian SF occurs in Africa, Europe, and Asia. The epidemiology is closely associated with the habitats of the vector *P. papatasi*, which is found throughout the Mediterranean, extending as far east as India and Transcaucasia.

Clinical Features

SF is an acute, self-limiting febrile illness normally transmitted by the bite of the sandfly. The disease was recognized in 1887, and in 1908 an Austrian military commission reproduced the disease in humans by inoculation of healthy individuals with blood obtained from patients in the first day of fever. The clinical manifestations of sandfly fever were studied beginning in 1944 by Dr. Albert Sabin in more than 100 cases of experimentally induced disease (127). Following intravenous inoculation, the incubation period is 1.5–3.5 days. SF is sudden in onset with fever, lasting 2–4 days, and characterized by frontal and retroorbital pain, headache, photophobia, generalized malaise, arthralgia, and low back pain. Anorexia, nausea, and, not infrequently, vomiting are associated with ill-defined abdominal distress. Approximately 65% of patients have fever over 102°F but not above 104.5°F. Fever above 100°F is seen in all patients who complain of a flu-like illness. The disease is self-limiting with complete recovery; no deaths have oc-

curred among thousands of clinically diagnosed cases.

Experimental Therapeutics: Clinical Trials

It has not been possible to conduct a clinical trial against Rift Valley fever; however, a controlled trial was conducted at USAMRIID against SF (Sicilian), which is useful in evaluating potential clinical efficacy of the drug against RVF. This model has been used at USAMRIID since 1964 to study the effects of viral infection in humans. Ribavirin was evaluated in a double-blind, placebo-controlled study of prophylactic efficacy in prevention of sandfly fever (Sicilian) virus infection in human volunteers (C. Macdonald, K. McKee, J. Huggins, and P. Canonico, unpublished observations). Twelve adult human volunteers were inoculated IV on day 0 with diluted human plasma containing sandfly fever (Sicilian) virus. Six subjects received oral ribavirin, 400 mg each 8 hr for a total of 1,200 mg/day beginning 1 day before infection and continuing for 8 days. Six subjects received placebo in an identical manner. Four of six placebo controls became ill with fever, chills, myalgia, prostration, and headache lasting 3 days. Serum chemistries were unaffected, but leukopenia (mean WBC = 2,870) and decreased platelet (mean = 140,000) were seen. All four had fever of greater than 100°F for 3 days, at the same time as clinical illness. During this time, sandfly antigen was detected by ELISA. Serum interferon levels were elevated in clinically ill patients. These four placebo-treated patients showed positive serological evidence of infection as demonstrated by specific IgM and IgG. All ribavirin treated subjects remained healthy without clinical signs during the course of the study, and five of six seroconverted. The failure to infect some subjects, as judged by seroconversion, is believed to be caused by the long storage of the inoculum

virus, which had lost infectivity. To provide meaningful comparisons, analysis concentrated on these patients with positive serological evidence in infection. All four seropositive placebo patients became ill, with fever, clinical signs and symptoms, and circulating viral antigen. All five seropositive ribavirin-treated patients remained asymptomatic during the entire study, providing evidence that ribavirin can prevent the infection by a bunyavirus in humans (128).

Flaviviruses

Many flavivirus infections produce an uncomplicated febrile disease in a significant percentage of patients; those that are capable of causing significant sequelae are covered in the section associated with those sequelae.

Dengue

Dengue causes a febrile illness in the vast majority of cases, but is capable of causing severe hemorrhagic disease under certain conditions. Dengue is reviewed in the section on hemorrhagic fevers.

Alphaviruses

Chikungunya Virus

Etiologic Agent

Chikungunya virus is an alphavirus, with properties similar to other members of the family. It is serologically related to o'Nyong-Nyong.

Epidemiology

Chikungunya virus is transmitted among monkeys and baboons by forest *Aedes* mosquitoes. Large epidemics of chikun-

gunya occur in urban and semiurban settings where the virus is transmitted by *Aedes aegypti*. Such epidemics can be explosive, involving, almost simultaneously, large populations. The geographic distribution is sub-Saharan Africa, India, and southeast Asia. Infections in southeast Asia have coincided with outbreaks of dengue from the same vector (1).

Clinical Features

Chikungunya is characterized by fever, rash, and arthritis. Following an incubation of 3–12 days, the disease presentation is strikingly abrupt, with intense pain in one or more joints, followed by high fever and myalgia. Rash develops on day 2–5 after onset and is maculopapular. The acute disease lasts 3–10 days, but convalescence may include prolonged joint swelling and pain lasting weeks to months (1).

Prevention and Treatment

No specific therapy is available.

DISCUSSION

Significant progress has been made in the development of antiviral chemotherapy for hemorrhagic fevers. Much of this success must be attributed to the good fortune of the discovery of ribavirin, because studies of over 100 close analogs have revealed few with similar or significantly increased antiviral activity in animal models (J.W. Huggins and USAMRIID Antiviral Drug Screening Program) and none is at a state of development to allow for clinical trials.

Intravenous ribavirin is currently an investigational new drug for hemorrhagic fever with renal syndrome and is not approved for general human use by the United States Food and Drug Administration (FDA). In clinical trials, ribavirin has

demonstrated therapeutic benefit against two hemorrhagic fevers, Lassa fever and HFRS. Animal models have demonstrated increased rates of survival and inhibition of viral replication for several hemorrhagic fever viruses belonging to the bunyavirus and arenavirus families. The weight of the evidence supports the merit of further studies of ribavirin in patients with Crimean-Congo hemorrhagic fever. It argues against a beneficial effect of the drug in dengue and yellow fever. The toxicity of ribavirin appears to be manageable and is fully reversible. It has not prevented treatment of hemorrhagic fever patients with effective levels of drug. Several new drugs, natural products and analogs of ribavirin, with improved therapeutic ratios are under evaluation, as are possible combinations of ribavirin or an analog with an interferon inducer to improve the overall efficacy. Such combinations may ultimately yield improved therapy, but are not yet ready for clinical trials.

The clinical trials necessary to establish new therapies for these viral diseases are particularly difficult for many reasons, including their predominant occurrence in the Third World where the medical infrastructure is much less developed and funding for health care is limited. Some of these diseases occur in an unpredictable fashion, making planning particularly difficult. Rapid and accurate diagnosis is also a requirement of any useful clinical trial. Despite these difficulties, given the impact of these diseases on the world community, additional clinical trials are clearly needed to achieve effective treatment of life-threatening diseases. Whenever possible, these trials should be prospective, blinded, and placebo-controlled to maximize the value of the results.

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